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Published in:

Proceedings of the 9th Symposium of the International Society for Veterinary Epidemiology and Economics, August 6-11 2000, Colorado, USA

Publication date:

2000

Document Version

Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):

Chriél, M. (2000). Interpretation of test results in eradication programmes using multiple sampling. In *Proceedings of the 9th Symposium of the International Society for Veterinary Epidemiology and Economics, August 6-11 2000, Colorado, USA*

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INTERPRETATION OF TEST RESULTS IN ERADICATION PROGRAMMES USING MULTIPLE SAMPLING.

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Since perfect diagnostic tests, i.e. test with 100 percent sensitivity and specificity are rarely adaptable for practical use in disease surveillance virtually all disease control programmes are faced with the problem of how to interpret the occurrence of single reactors in herds declared free of the corresponding disease.

Aleutian Disease (AD) is characterised by a long incubation period and lack of pathognomonic symptoms (An and Ingram, 1977). The causative agent, a parvo virus is extremely stable in the environment.

A voluntary AD eradication programme was initiated in Denmark in 1976 following the development of a specific serologic test (counter immunoelectrophoresis) applicable for mass screening purposes (Cho and Ingram, 1972). The programme implied whole-herd testing and removal of reactors. A herd was declared free of the disease after two consecutive tests of all animals in the herd with minimum nine months and maximum 18 months in-between without detection of reactors. The maximum number of samples examined during one year was 3.9 million (1988). During the last five years approximately 2 million samples have been examined annually. The programme is executed and financed by the Danish Fur Breeders' Association.

Initially, all Danish mink farms were infected with AD virus, the average within-herd prevalence being around 60%. The first three farms were declared AD-free in 1979. Twenty years later more than 95% of the 2300 Danish mink farms are free.

The current programme involves two routine testings annually in all AD-free farms, one during summer when 10% of the breeding animals are sampled, and one in the winter (20% of the breeding animals to be sampled). Additional testing is required for AD-free farms located in AD-endemic areas, i.e. all breeding animals must be tested in the winter period. The average farm size is approximately 800 breeders. When single reactors (up to 10 positive samples) are detected in AD-free farms, blood samples are taken from the reactors and sent to the laboratory for re-examination. Three different re-testing procedures are employed.

This paper will focus on the practical interpretation of the disease classification of farms having single reactors and the prognostic value of re-testing procedures.

Materials and Methods

The analyses which follow are based on the test results from all farms declared AD-free during the period 1996-1998. The probabilities of a positive test result have been calculated separately for each of the four major regions in Denmark.

The impact of the number of single reactors on the classification of the AD-status of the farms is stated for the period 1995-98.

Finally, results from re-test of all single reactors detected in the period 1995-1998 from the above mentioned farms are evaluated by using the next years herd testing as "Golden Standard".

Results

The results from the voluntary programme are as follows:

Year	Region	Total number of farms	Number (%) of AD-free farms	Total number of single reactors	Probability of a reactor per 1000 tests
1996	1	165	143 (87)	10	0,043
	2	941	766 (81)	164	0,110
	3	791	754 (95)	34	0,107
	4	565	513 (91)	52	0,052
1997	1	169	152 (90)	2	0,150
	2	967	810 (84)	157	0,140
	3	812	784 (97)	30	0,035
	4	575	532 (93)	42	0,063
1998	1	171	158 (92)	4	0,099
	2	948	763 (80)	73	0,198
	3	818	799 (98)	11	0,028
	4	577	542 (94)	22	0,087

Table 1: The test results from the Danish Aleutian Disease surveillance programme, 1996-98.

Year	AD-status in farms following re-testing of single reactors	No. of single reactors per farm			
		1	2	3	4 - 10
1995	AD-infected	23 ¹	10	11	21
	AD-free	29	7	3	2
	P(AD-infected no. of single reactors)	0.32	0.59	0.79	0.91
1996	AD-infected	21	7	7	20
	AD-free	35	9	1	2
	P(AD-infected no. of single reactors)	0.38	0.44	0.88	0.91
1997	AD-infected	30	11	2	3
	AD-free	26	7	0	1
	P(AD-infected no. of single reactors)	0.54	0.61	1.00	0.74
1998	AD-infected	40	12	10	15
	AD-free	34	5	1	1
	P(AD-infected no. of single reactors)	0.54	0.71	0.91	0.94

¹ Number of farms.

Table 2. Classification of farms with single reactors based on the results from re-testing of the reactors.

AD-status of farm based on the results of re-testing	Number of single reactors re-tested			
	1	2	3	4-10
AD-infected	0,38	0,56	0,63	0,82
AD-free	0,17	0,30	NA	0,40

Table 3: The probability of detecting one or more reactors in farms in connection with next herd testing, according to classification with respect to AD-status based on the results of re-testing of single reactors.

Using the following year's herd testing result as the Golden Standard for evaluating the validity of re-testing, the procedure has a sensitivity of 0.87 and a specificity of 0.54 when applied at the individual animal level.

Discussion and conclusion.

It would be tempting to ascribe the occurrence of single reactors in AD-free farms to false positive test results. However, analysis of data obtained from the Danish eradication programme have shown an association between regional herd prevalence and the number of single reactors, implying that single reactors tend to cluster geographically (Table 1). The reoccurrence of single reactors in about half of the affected farms also indicate that the majority of positive test results are caused by infection with AD virus. Therefore, it appears reasonable to assume that the specificity of the test employed in the programme is fairly close to 1.

The specificity of a diagnostic test employed for disease surveillance in an eradication programme will have a huge impact on farm specificity because the test often is applied to thousands of samples.

Frequent detection of single reactors in herds where no reactors are found during subsequent testing lead the farmer to believe that the originally detected single reactor(s) occurred due to a false positive reaction, thereby bringing the routine testing programme into discredit.

Only AD-free farms are allowed to sell breeding stock. Since some farms sell more than 10.000 minks annually misclassification of a farm with respect to its AD-status will have a significant negative impact on the economy. Therefore, it was originally decided that all single reactors be re-tested in order to sort out false positives. Unfortunately, the discrepancies between the routine test results and the results from re-testing have given rise to much debate. Fluctuating levels of detectable antibodies are one reason for the discrepancies. Another reason may be the failure to identify correctly the single reactor or a deliberate sampling of a non-reactor. The procedures carried out in connection with detection of single reactors have therefore been modified. The results from re-testing are no longer used for classification of farms but only for prognostic purposes.

The risk of a farm being classified AD-infected increases with the number of single reactors detected during routine testing (Table 2). Furthermore, the impact of parallel interpretation of the results from re-testing in 1997 increased the risk in 1997 and 1998 for farms having only one or two reactors whereas the risk for farms with 3-10 single reactors being classified as infected was unchanged.

Although it could be argued that re-testing of single reactors has no immediate utility to the farmer the procedure does have prognostic value because the risk of having reactors at next herd testing depends both on the classification based on the re-test results and the number of single reactors (Table 3).

Lack of test sensitivity is often a severe problem at the end of an eradication programme due to the risk of breeding animals being commercially distributed from newly infected farms but still not detectable in connection with routine surveillance. If all single reactors are assumed to be true positive it can be concluded that although the test sensitivity in newly infected farms is fairly low, the herd sensitivity will be high due to the large number of animals being tested.

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